

Claims

1. A composition for treating a bacterial biofilm, comprising a first bacteriophage that is capable of infecting a bacterium within said biofilm, and a first polysaccharide lyase enzyme that is capable of degrading a polysaccharide within said biofilm.
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2. A composition according to Claim 1, further comprising a pharmaceutically-acceptable antimicrobial agent, preferably an antibiotic or a defensin.
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3. A composition according to Claim 1 or Claim 2, further comprising a DNase.
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4. A composition according to any preceding claim, further comprising a second polysaccharide lyase, wherein the first and second polysaccharide lyase are different.
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5. A composition according to any preceding claim, wherein the first polysaccharide lyase is encoded by the bacteriophage.
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6. A composition according to any previous claim, wherein the bacteriophage encodes one or more of a pharmaceutically-acceptable antimicrobial agent, a DNase, or a second polysaccharide lyase that is different from the first polysaccharide lyase.
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7. A composition according to any previous claim, comprising a second bacteriophage, which is different from the first bacteriophage, and wherein the second bacteriophage optionally encodes a second polysaccharide lyase.
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8. A composition according to any previous claim, comprising a second pharmaceutically-acceptable antimicrobial agent.
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9. A composition according to any previous claim, wherein the biofilm is a lung biofilm.

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10. A composition according to any preceding claim, wherein the biofilm comprises an opportunistic bacterium, preferably *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*.

5 11. A composition according to any preceding claim, wherein the phage is a GH phage, preferably GH4 (ECACC Accession No. 02121203), GH6 (ECACC Accession No. 02121202), GH13 (ECACC Accession No. 02121201), or GH14 (ECACC Accession No. 02121204).

10 12. A composition according to any preceding claim, wherein the first and/or second polysaccharide lyase is an alginic acid lyase.

13. Use of a composition according to any previous claim for the manufacture of a medicament for treatment of a biofilm.

15 14. Use according to Claim 13, wherein the biofilm is a lung biofilm in a cystic fibrosis patient.

20 15. Use according to Claim 13 or 14, wherein the medicament is to be administered in more than one separate dose.

16. Use according to Claim 15, wherein the medicament is to be administered in at least three separate doses.

25 17. Use according to any of Claims 13-16, wherein following administration the bacterial cell count of the biofilm is reduced by at least one log.

18. Use according to any of Claims 13-17, wherein the bacterial cell count of the biofilm is reduced by at least three logs.

30 19. Use according to any of Claims 13-18, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to the first polysaccharide lyase.

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20. Use according to any of Claims 13-19, wherein the first bacteriophage and the first polysaccharide lyase are to be administered as part of a combined therapy regimen with a pharmaceutically-acceptable antimicrobial agent, and wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to said pharmaceutically-acceptable antimicrobial agent.

5 21. Use according to any of Claims 13-20, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second polysaccharide lyase that is different from the first polysaccharide lyase.

10 22. Use according to any of Claims 13-21, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second bacteriophage that is capable of infecting a bacterium within the biofilm, wherein said second bacteriophage is different from the first bacteriophage.

15 23. A bacteriophage comprising a heterologous gene encoding a first polysaccharide lyase enzyme.

20 24. A bacteriophage according to Claim 23, wherein the bacteriophage is capable of infecting a bacterial species or strain present in a biofilm.

25 25. A bacteriophage according to Claim 23 or Claim 24, wherein the polysaccharide lyase degrades a polysaccharide component of a biofilm.

25 26. A bacteriophage according to any of Claims 23-25, wherein the bacteriophage infects an opportunistic bacterium, preferably *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*.

30 27. A bacteriophage according to any of Claims 23-26, wherein the bacteriophage is a GH phage encoding a first polysaccharide lyase, preferably GH4 (ECACC Accession No. 02121203) encoding a first polysaccharide lyase, GH6 (ECACC Accession No. 02121202) encoding a first polysaccharide lyase, GH13 (ECACC Accession No. 02121201) encoding a first polysaccharide lyase, or GH14 (ECACC Accession No. 02121204) encoding a first

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polysaccharide lyase.

28. A composition according to any of Claims 1-12, wherein the first bacteriophage is a bacteriophage according to any of Claims 23-27.

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29. A composition according to Claim 28, further comprising a second bacteriophage according to any of Claims 23-27, wherein the first bacteriophage and second bacteriophage are different.

10 30. A composition according to any of Claims 1-12 or 28-29 in the form of an aerosol formulation, comprising one or more of an excipient, surfactant, and/or propellant.

15 31. Use of a bacteriophage according to any of Claims 23-27 or a composition according to any of Claims 28-30 for the manufacture of a medicament for treating a biofilm, preferably for treating a biofilm that results from an opportunistic bacterial infection.

20 32. Use according to Claim 31, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to the first polysaccharide lyase.

25 33. Use according to Claim 31-32, wherein the first bacteriophage and the first polysaccharide lyase is to be administered as part of a combined therapy regimen with a pharmaceutically-acceptable antimicrobial agent, and wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to said pharmaceutically-acceptable antimicrobial agent.

30 34. Use according to any of Claims 31-33, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second polysaccharide lyase that is different from the first polysaccharide lyase.

35. Use according to any of Claims 31-35, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second

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bacteriophage that is capable of infecting a bacterium within the biofilm, wherein said second bacteriophage is different from the first bacteriophage.

36. A method of making a modified bacteriophage capable of degrading a biofilm

5 comprising:-

a) selecting at least one gene encoding a polysaccharide lyase enzyme that degrades a polysaccharide within said biofilm;

b) selecting a bacteriophage that is capable of infecting a bacterial species or 10 strain residing within the biofilm; and

c) introducing at least one of the genes selected in step a) into the bacteriophage nucleic acid.

37. A method according to Claim 36, wherein the bacteriophage is selected from

15 the group consisting of GH4 (ECACC Accession No. 02121203), GH6 (ECACC Accession No. 02121202), GH13 (ECACC Accession No. 02121201), or GH14 (ECACC Accession No. 02121204); or a bacteriophage having accession No. ATCC 12055-B1, ATCC 12055-B2, ATCC 12055-B3, 20 ATCC 14205-B1, ATCC 14206-B1, ATCC 14207-B1, ATCC 14209-B1, ATCC 14210-B1, ATCC 14211-B1, ATCC 14212-B1, ATCC 14213-B1, ATCC 14214-B1, ATCC 15692-B2, ATCC 15692-B3, ATCC 25102-B1, ATCC BAA-26-B1, ATCC BAA-27-B1, ATCC BAA-28-B1, ATCC BAA-28-B2, ATCC BAA-29-B1, ATCC BAA-30-B1, ATCC BAA-31-B1, ATCC BAA-47-B1, ATCC BAA-79-B1, 25 ATCC BAA-81-B1, and ATCC BAA-81-B2.

25 38. A method according to Claim 36-37, wherein the method further comprises the step of testing the efficacy of the modified bacteriophage against the biofilm *in vitro*.

30 39. A method according to any of Claims 36-38, wherein the bacteriophage specifically infects an opportunistic bacterium, preferably *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*.

40. A method according to any of Claims 36-39, wherein said at least one gene

encodes an alginate lyase.

41. A method of identifying a bacteriophage for use in treatment of a biofilm-associated microbial infection, which comprises:-
 - 5 a) identifying a bacteriophage that is capable of infecting a bacterial species or strain with said biofilm; and
 - b) confirming that said bacteriophage encodes a polysaccharide lyase that degrades a polysaccharide within the biofilm.
- 10 42. A method of treating a biofilm infection, which method comprises:-
administering to a patient a composition according to any of Claims 1-12 or 28-30, or a bacteriophage according to any of Claims 23-27.
- 15 43. A method according to Claim 42, wherein the composition or bacteriophage is administered in more than one separate dose.
44. A method according to Claim 42 or 43, wherein the composition or bacteriophage is administered in at least three separate doses.
- 20 45. A method according to any of Claims 42-44, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to the first polysaccharide lyase.
46. A method according to any of Claims 42-45, wherein the first bacteriophage and the first polysaccharide lyase are administered as part of a combined therapy regimen with a pharmaceutically-acceptable antimicrobial agent, and wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to said pharmaceutically-acceptable antimicrobial agent.
- 25 30 47. A method according to any of Claims 42-46, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to a second polysaccharide lyase that is different from the first polysaccharide lyase.
48. A method according to any of Claims 42-47, wherein the first bacteriophage

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is administered prior to, simultaneously with, or subsequent to a second bacteriophage that is capable of infecting a bacterium within the biofilm, wherein said second bacteriophage is different from the first bacteriophage.

5 49. A method according to any of Claims 42-48, wherein administration is to the site of infection.

50. A method according to any of Claims 42-49, wherein administration is to a surface selected from a lung, a gastrointestinal tract, a catheter, an intra-
10 vascular device, a prosthetic device, or a dental implant.